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REVIEW

Molecular control of autonomous embryo and endosperm development

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Abstract Precocious seed development is usually prevented by a series of mechanisms that ensure seed production results from double fertilization. These events are circumvented in natural apomictic plant species that reproduce clonally through seed. Recent advances in molecular genetics using mutagenic approaches in model sexual plant species, such as *Arabidopsis* and *Zea mays*, have revealed some of the mechanisms that prevent such precocious seed development. An understanding of these mechanisms may lead to the development of techniques that will allow future crop plant species exhibiting hybrid vigor to be engineered such that their complex genomes can be fixed indefinitely, thereby maintaining high yields. Our current understanding of the mechanisms underlying the processes of reproductive development is discussed in this review.

Keywords Apomixis · Endosperm · Egg activation · Parthenogenesis

Introduction

Seed development is central to the life cycle of higher plants and played a crucial role in their evolutionary success. The developmental program of the seed is initiated by double fertilization and requires the coordinated development of the maternal seed coat together with the two fertilization products, the embryo and endosperm (reviewed in Berger et al. 2006). For double fertilization to take place, the pollen tube that carries the two sperm cells enters the embryo sac through the micropyle guided by signals from the synergids (for a detailed description see Punwani and Drews 2008; Higashiyama and Hamamura 2008, this issue). The pollen tube penetrates the degenerating synergid cell, releasing the sperm cells contained within—a process that is under the control of the embryo sac (Huck et al. 2003, Rotman et al. 2003; Escobar-Restrepo et al. 2007). One sperm cell fuses with the egg cell to produce the zygote and the other fuses with the central cell to produce the endosperm (for a detailed description see Martón and Dresselhaus 2008, this issue). This double fertilization event triggers seed development.

A series of checkpoints throughout reproductive development ensures that viable seed depends on double fertilization. Precocious seed development can results, however, when these developmental checkpoints are circumvented. The production of precocious seed occurs naturally in more than 400 species in a process known as apomixis. Apomixis refers to the asexual reproduction through seeds, resulting from: (1) an absent or aberrant meiosis (apomeiosis) (2) the fertilization-independent initiation of embryogenesis (parthenogenesis) and (3) the formation of functional endosperm either autonomously or after fertilization (pseudogamy) (reviewed in Grimanelli et al. 2001; Koltunow and Grossniklaus 2003; Spielman et al. 2003). The molecular mechanisms allowing such precocious seed development have been difficult to determine in apomictic species. However, recent advances examining developmental checkpoints have progressed

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significantly using model plant species such as *Zea mays* (maize) and *Arabidopsis thaliana*. Using these sexual species, a variety of genetic approaches have been employed that led to the identification of mutants showing elements of precocious seed development, such as parthenogenesis or the production of autonomous endosperm. These mutations reveal some of the molecular mechanisms that tightly control sexual seed production, the deregulation of which creates phenotypes reminiscent of the components of apomixis.

Egg activation and induction of embryogenesis

The fertilization of the egg cell triggers its activation and initiates embryogenesis. In animals, the egg cell is arrested at a specific stage of meiosis. Fertilization leads to the release of this arrest, a process referred to as "egg activation''. In all animal species that have been investigated to date, fertilization triggers a dramatic increase in the intracellular Ca^{2+} concentration, leading to a resumption of meiosis and the formation of pronuclei (reviewed in Miyazaki and Ito 2006). The increased wave of Ca^{2+} which starts at the site of sperm entry and propagates across the entire egg cell, is induced by a sperm-derived factor. Importantly, the increase in Ca^{2+} through the administration of Ca^{2+} ionophores, is sufficient to trigger parthenogenetic development of animal egg cells (Steinhardt and Eppel 1974; Uranga et al. 1996), suggesting that Ca^{2+} can induce all of the downstream signaling events required to initiate embryogenesis.

An increase in intracellular Ca^{2+} similar to that observed in animal zygotes has also been described after in vitro fertilization of maize gametes (Antoine et al. 2000), (for a detailed description see Feijo 2008, this issue). However, while certain aspects of egg activation are affected by the administration of pharmacological agents that modulate Ca^{2+} influx (Antoine et al. 2001), an increase in intracellular Ca^{2+} concentration is not sufficient to trigger parthenogenesis in plants. Thus, the exact mechanisms that prevent embryo development in the absence of fertilization and those that trigger it after fertilization are unknown, and to date no mutants have been identified that prevent egg activation after fertilization.

While the molecules involved in zygotic embryo activation are currently not known, several publications reported that the over-expression of certain transcription factors can induce somatic embryogenesis in young seedlings. Ubiquitous over-expression of the transcription factors BABYBOOM (BBM), LEAFY COTYLEDON1 (LEC1) and LEC2, and WUSCHEL (WUS) can induce the formation of somatic embryos in seedlings or roots (Boutilier et al. 2002; Lotan et al. 1998; Stone et al. 2001; Zuo et al. 2002). These genes, however, seem unlikely to be directly involved in zygotic embryo initiation since during wild-type development, they do not appear to be expressed in the egg cell or the zygote (Mayer et al. 1998; Boutilier et al. 2002; V. Gagliardini and U. Grossniklaus, unpublished data). The production of somatic embryos resulting from the over-expression of these genes may be caused by stress, which has been shown to induce somatic embryogenesis in *Arabidopsis* seedlings (Ikeda-Iwai et al. 2003) and is also associated with other somatic embryogenesis systems (Nolan et al. 2006). Cellular stress may lead to the de-differentiation of specific cells that acquire embryonic identity in response to other signals derived from surrounding tissues, such as auxin (Gallois et al. 2004). This hypothesis is consistent with the finding that not all cells can respond to the over expression of such transcription factors (Boutilier et al. 2002; Stone et al. 2001; Zuo et al. 2002) and somatic embryos often form in regions where high levels of auxin accumulate, such as lateral root primordia, the root apex and the cotyledons of young seedlings (Ni et al. 2001). The competence of juvenile tissue to de-differentiate and acquire embryonic identity at early stages of development in response to stress may provide a mechanism by which a stressed seedling is rescued through somatic embryogenesis. Later in development, such stress will no longer result in the initiation of somatic embryos, but in early flowering, providing the opportunity for survival through seeds. Although stress is not usually associated with zygotic embryogenesis, an increase in ethylene synthesis and endogenous auxin levels is observed (Ribnicky et al. 2002; Mòl et al. 2004).

The observations described above provided the impetus to mis-express selected transcription factors in the egg cell or ovule; however, these appealing experiments have as yet failed to initiate embryo development in the context of the ovule (L. Brand, U. Grossniklaus and M. Curtis, unpublished results). To date, only one factor, the *Arabidopsis* SOMATIC EMBRYOGENESIS RECEPTOR KINASE1 (*SERK1*), which increases the embryonic potential of cultured *Arabidopsis* cells, has been shown to be expressed in regions of developing wild-type ovules, including the egg cell (Hecht et al. 2001). The appropriate signal required to stimulate embryonic potential in the egg cell, however, remains elusive.

Autonomous endosperm development

Usually, fertilization triggers endosperm and embryo development in flowering plants, however, several mutants have been isolated in *Arabidopsis* that allow elements of endosperm and/or embryo development to occur in the absence of fertilization. These mutants are known as

the fertilization-independent seed (*fis*) class of mutants (reviewed in Grossniklaus et al. 2001) and include *medea* (*mea*) (Grossniklaus et al. 1998a; Kinoshita et al. 1999), *fis2* (Chaudhury et al. 1997; Luo et al. 1999), *fertilization-independent endosperm* (*fie*) (Ohad et al. 1996; Ohad et al. 1999) and *Arabidopsis multisupressor of ira1* (*msi1*) (Köhler et al. 2003a; Guitton et al. 2004), encoding a p55-like histone-binding protein, which is also a component of several other multiprotein complexes (Hennig et al. 2003). The *fis* class mutants identify genes encoding proteins similar to those first discovered in *Drosophila*, forming what is known as *Polycomb* group (PcG) complexes. There are three distinct types of PcG complexes in *Drosophila*: the *Polycomb* Repressive Complex 1 (PRC1) and PRC2, as well as the *polyhomeotic* Repressive Complex (*pho*RC) (reviewed in Müller and Kassis 2006). These complexes play a role in the maintenance of target gene repression throughout development (Ringrose and Paro 2004). In *Arabidopsis*, only genes encoding PRC2 components have been characterized and there is evidence for several distinct PRC2-like complexes that play various roles during development (Grossniklaus and Paro 2007).

The FIS–PRC2 complex specifically maintains the repression of genes involved in cell proliferation during reproductive development (reviewed in Köhler and Grossniklaus 2002; Guitton and Berger 2005). All mutations in *FIS* class genes show two distinct phenotypes. If fertilized, seeds derived from a *fis* mutant gametophyte show an aberrant proliferation of embryo and endosperm, and eventually abort irrespective of the paternal contribution. In other words, these mutants display gametophytic maternal-effect embryo lethality. In addition, in the absence of fertilization, all *fis* mutants initiate endosperm development from the central cell, while simultaneously triggering fruit development (Fig. 1b). Although most apomicts require the fertilization of the central cell, autonomous endosperm also develops in several apomictic species, such as *Taraxacum* spp. and *Hieracium* spp. (van Dijk et al. 2004; Koltunow et al. 1998, respectively). Marker analyses, using the *Arabidopsis FIS*-class promoters to drive *β*-glucuronidase (GUS) reporter gene expression, have shown that the expression profiles for these genes are broadly similar between apomictic and sexual *Hieracium* species (Tucker et al. 2003). At present it is not known whether the *FIS* class genes play any functional role in autonomous endosperm development in natural apomicts.

Genomic imprinting during seed development

The balance between maternal and paternal (m:p) genome contributions in the endosperm is disturbed in both pseudogamous and autonomous apomicts. In sexual maize, for example, normal endosperm only develops with a 2m:1p genome ratio, whereas all other ratios lead to seed abortion. In many apomicts the 2m:1p genome ratio is maintained through specific adaptations that alter male or female gametophyte development or double fertilization (Grossniklaus et al. 1998b; Grossniklaus 2001; Koltunow and Grossniklaus 2003). In some pseudogamous apomicts, fertilization of the unreduced central cell with a reduced sperm cell creates an imbalance with genome ratios different from 2m:1p (Grimanelli et al. 1997; Quarin 1999), and in autonomous apomicts even an imbalance of 4m:0p is tolerated. These seed abortion phenotypes, which are circumvented in some apomictic species, are interpreted to result from gene dosage effects (Birchler 1993; Dilkes and Comai 2004). Genes that are regulated by genomic imprinting show allelelic expression differences that are dependent on the parental origin of the allele. Since genomic imprinting affects gene dosage in a parent-of-origin-dependent way, it is likely to play a role in such dosage effects (Grossniklaus et al. 1998b; Haig and Westoby 1991; Vinkenoog et al. 2001).

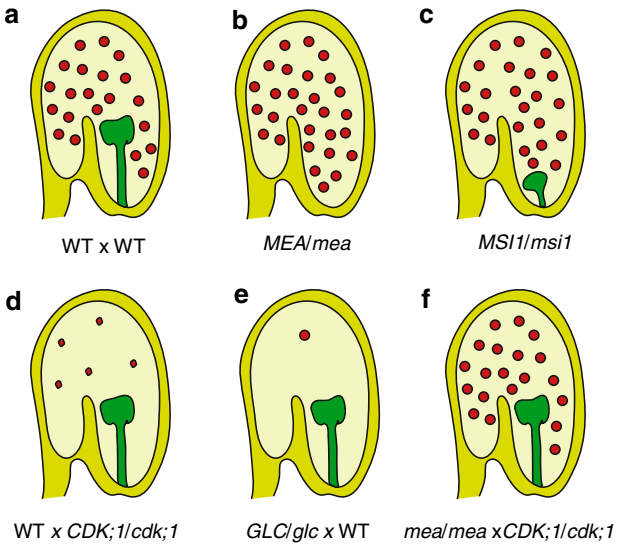

Fig. 1 **a** Wild-type (WT) seed development after WT fertilization of a WT embryo sac (WT × WT), showing a globular stage diploid embryo (*dark green*) surrounded by developing triploid endosperm (*red*). **b** Mutant *mea* seed development showing fertilization-independent (autonomous) diploid endosperm (*red*) in the absence of an embryo. **c** Mutant *msi1* seed development showing fertilization-independent (autonomous) diploid endosperm (*red*) surrounding an irregular parthenogenetic embryo (*dark green*). **d** Mutant seed development after a WT embryo sac was fertilized with a mutant *cdk;1* pollen showing a globular diploid embryo (*dark green*) surrounded by remnants of diploid endosperm nuclei and cytoplasm. **e** Mutant seed development after a *glc* mutant embryo sac was fertilized by WT pollen showing a globular diploid embryo (*dark green*) developing in the absence of central cell fertilization and endosperm. **f** Mutant viable seed development after a *mea* embryo sac was fertilized with *cdk;1* pollen showing a globular diploid embryo (*dark green*) surrounded by developing diploid endosperm

The maternal-effect seed abortion observed in the *fis* class mutants could result from the disruption of maternally derived gene products stored in the egg and/or central cell, which are required later for seed development, or they may result from a dosage effect where one wild-type paternal and two mutant maternal alleles may not be sufficient for normal endosperm formation. Alternatively, *fis* maternal effects may result from regulation by genomic imprinting, where the genes are transcribed exclusively from the maternally inherited alleles after fertilization (Grossniklaus et al. 1998a). Genomic imprinting can be demonstrated by the detection of different levels of maternally and paternally derived transcripts after fertilization, indicating that the two alleles have distinct epigenetic transcription states, even though they are in the same nucleus. However, if a gene is already transcribed prior to fertilization, the difference in transcript levels after fertilization could simply stem from maternally produced transcripts that were generated prior to fertilization. Therefore, it is very important to demonstrate active transcription in the fertilization products, for instance through the detection of nascent transcripts in the nucleus by in situ hybridization (discussed in Grossniklaus 2005; Baroux et al. 2007).

The *FIS* class genes are all expressed prior to fertilization (Grossniklaus et al. 1998a; Ohad et al. 1999; Luo et al. 2000; Köhler et al. 2003a) and *FIE* as well as *MSI1* are biallelically expressed during seed development (Yadegari et al. 2000; Leroy et al. 2007). In contrast, for *MEA* and *FIS2* only maternally derived transcripts could be detected after fertilization (Vielle-Calzada et al. 1999; Kinoshita et al. 1999; Jullien et al. 2006a). Since both *MEA* and *FIS2* are already transcribed prior to fertilization and their quantitative expression level decreases during seed development (Baroux et al. 2006), a demonstration of active transcription after fertilization is essential to demonstrate genomic imprinting. For *MEA*, the maternal effect was unambiguously shown to be due to genomic imprinting through the detection of nascent transcripts in endosperm nuclei (Vielle-Calzada et al. 1999). While the regulation of *FIS2* (Jullien et al. 2006a) strongly argues for genomic imprinting at this locus as well, evidence that *FIS2* is actively transcribed after fertilization has not yet been demonstrated. This is also true for *FWA*, another gene for which maternal expression during seed development has been demonstrated, but whose role during seed development remains unclear (Kinoshita et al. 2004).

Genomic imprinting provides a mechanism by which genes are expressed in a parent-of-origin-specific manner. The evolution of this phenomenon may be explained by the parental conflict theory, which suggests that the interests of the parents differ with regard to the allocation of resources from the mother to the progeny (Haig and Westoby 1989). Paternal genes are proposed to promote offspring growth, while maternal genes are proposed to reduce growth, thus distributing resources evenly among all progeny. Genomic imprinting, which in this form has only been described in mammals and seed plants, seems to involve either DNA methylation and/or histone modification, in both of these distant lineages (reviewed in Feil and Berger 2007). The silencing mark used to maintain a silent paternal *FIS2* allele during gametogenesis, and thereafter during endosperm development, depends on cytosine methylation through the action of the DNA *METHYLTRANSFERASE 1* (*MET1*) (Jullien et al. 2006a), which is responsible for the maintenance of CpG methylation. This mark of CpG methylation is believed to be removed maternally in the female gametophyte by the direct or indirect action of DEMETER (DME) (Choi et al. 2004; Xiao et al. 2003; Jullien et al. 2006a)—a DNA-glycosylase that removes methylated cytosines and replaces them with unmethylated cytosines (Gehring et al. 2006; Morales-Ruiz et al. 2006). This mechanism would explain the specific expression of the maternal *FIS2* allele. Similarly, the *MEA* locus is activated maternally prior to fertilization, probably by a similar mechanism involving DME, which acts as a direct or indirect activator of the maternal *MEA* allele (Choi et al. 2002: Gehring et al. 2006). The maintenance of a paternally silent *MEA* allele in pollen and vegetative tissue is not dependent on DNA methylation, since it is not activated in pollen or vegetative tissue in *met1* loss-of-function mutants (Jullien et al. 2006). Interestingly, mutations in the *DECREASE IN DNA METHYLATION1* (*DDM1*) locus act as zygotic suppressors of *mea* in the presence of a wild-type *MEA* allele (Vielle-Calzada et al. 1999). DDM1 is not a DNA methyltransferase, rather a member of the SWI/SNF family of adenosine triphosphate-dependent chromatin remodeling proteins. The function of DDM1 is to modify DNA methylation and plays a role that links DNA methylation to histone modifications (Gendrel et al. 2002). In animals, the PRC2 complex itself modifies histones by methylating histone H3 at K27 (Czermin et al. 2002; Müller et al. 2002). In vegetative *Arabidopsis* tissues, a PRC2-like complex containing FIE and SWINGER (SWN) or CURLY LEAF (CLF), two homologs of MEA, mediate the silencing of *MEA* by methylating histone H3 at K27 in specific regions of the *MEA* promoter (Jullien et al. 2006b). In the male gametophyte, however, silencing is maintained by a different, largely unknown PcG complex, which includes FIE. During seed development, after fertilization, the paternal *MEA* allele remains silent. The maintenance of this silencing depends on the FIS-PRC2 complex containing MEA itself (Baroux et al. 2006; Gehring et al. 2006; Jullien et al. 2006b). Moreover, MEA represses the maternal *MEA* allele around fertilization, a function that is independent of other FIS–PRC2 components (Baroux et al. 2006).

Lack of *MEA* activity in the female gametophyte causes precocious seed development, allowing the endosperm to initiate, prior to fertilization and ultimately resulting in the abortion of these seed-like structures. This phenotype suggests that MEA is not only responsible for auto-repression, but also for the repression of other downstream target genes. Recently, some of these target genes have been identified including *PHERES1* (*PHE1*), a type I MADS-box gene expressed in a parent-of-origin-dependent manner (Köhler et al. 2003b). Here, the FIS-PRC2 has been shown to interact directly with the promoter of *PHE1* and is responsible for the down-regulation of the *PHE1* maternal allele (Köhler et al. 2005), accumulating H3K27 tri-methylation marks in the maternal *PHE1* locus as well as, to a lesser extent, H3K9 dimethylation marks (Makarevich et al. 2006). Interestingly, methylation of H3K27 and H3K9 have been implicated in the PcG-dependent silencing of *FLOWERING LOCUS C* (*FLC*), where it is thought that H3K27 methylation stimulates the methylation of H3K9 (Bastow et al. 2004). Although the precise function of *PHE1* is not known, mis-expression analysis has shown that it is chiefly responsible for the *mea* seed-abortion phenotype (Köhler et al. 2003b). This phenotype is suppressed in inbred *ddm1* mutants. DDM1 plays a role in the methylation of histone H3K9 (Gendrel et al. 2002). Changes in histone methylation from H3K9 to H3K4 correlate with the activation of gene expression (reviewed by Kouzarides et al. 2002). Interestingly, it has been shown that methylation at H3K9 and H3K27 is required for the recruitment of a methyltransferase, CHROMOMETHYLASE3 (CMT3) (Lindroth et al 2004), which is responsible for CpNpG methylation. Such global changes in chromatin structure in the *ddm1* mutant may not have a direct effect on the *MEA* locus, but may affect the expression of the downstream target gene *PHE1*, resulting in the suppression of the seed abortion phenotype. These effects are not straightforward, since the seed abortion phenotype is only alleviated in *mea* and *fis2* mutants by *ddm1*, but not in *fie* and *msi1* mutants. These differences may be due to the role these proteins play in other processes.

Spontaneous and Induced Parthenogenesis

MEA functions specifically during seed development, while *MSI1* and *FIE* are also active during later stages in development. MSI1, for example, plays a role in the chromatin assembly factor complex CAF1 that is required for the maintenance of the shoot and root apical meristems (Kaya et al. 2001). These additional functions may account for the capacity of *msi1* mutant seed to develop non-viable haploid embryos (Fig. 1c) and diploid endosperm without fertilization (Guitton and Berger 2005), and allow the spontaneous development of diploid endosperm after egg-cell fertilization (Köhler et al. 2003a). The haploid embryos derived from *msi1* mutant egg cells develop into irregular structures consisting of a maximum of 20 cells (Guitton and Berger 2005). Nevertheless, this phenotype clearly illustrates that the egg cell has the potential for parthenogenesis and that egg activation can be triggered in *msi1* mutants. Indeed, many plants can occasionally produce parthenogenetic haploid progeny (reviewed in Lacadena 1974). While such events are rather rare in most species, they can occur at frequencies as high as 0.1%, in maize (Chase 1969).

The fact that both embryos and endosperms can develop autonomously strongly suggests that the requirement for double fertilization can be relaxed. The second fertilization event appears necessary only to prevent the endosperm from developing in the absence of the embryo. Supporting evidence for this hypothesis comes from the *cyclin-dependent-kinase1* (*cdka;1*) mutant of *Arabidopsis* that produces pollen with only one sperm cell. This sperm cell exclusively fertilizes the egg cell and not the central cell (Nowack et al. 2006), providing a means by which to uncouple the double fertilization event. In these ovules, seed abortion takes place about 3 days after pollination, when the embryos produced are at the early globular stage surrounded by an under-developed endosperm (Fig. 1d). The recent report of the *glauce* (*glc*) mutant in *Arabidopsis* also supports the notion that early embryogenesis can be uncoupled from endosperm development (Fig. 1e). In seeds derived from *glc* mutant female gametophytes, the embryo reaches the globular stage in the complete absence of central cell fertilization and endosperm formation (Ngo et al. 2007).

Significantly, when *mea* mutant plants, capable of further autonomous endosperm development, are crossed with *cdka1* mutant pollen, embryogenesis proceeds to completion with only a diploid endosperm, producing a viable, albeit smaller seed (Fig. 1f). This finding suggests that genomic imprinting in the endosperm can be bypassed to allow seed development. In fact, this is exactly what occurs in apomicts with autonomous seed development, in which no paternal genome is required for normal endosperm development (Koltunow and Grossniklaus 2003).

The ability to uncouple the double fertilization events comes as no surprise when considering apomictic plants. In hybrids derived from crosses between maize cultivars and apomictic *Tripsacum dactyloides*, which reproduces asexually through seed, precocious embryo development initiates prior to fertilization, producing a pro-embryo of 8–32 cells, i.e. up to five divisions can occur (Grimanelli et al. 2003). Again, without endosperm, seed development is aborted. However, apomictic grasses resolve this problem by producing sexually derived endosperm (pseudogamy).

This allows the re-initiation of parthenogenetic embryo development. Importantly, evidence from sexual model plants suggests that early seed development is largely under maternal control (Vielle-Calzada et al. 2000). Although there is a basal level of transcription of paternally inherited alleles (Baroux et al. 2001), and not all paternally inherited alleles are silent (Weijers et al. 2001), the activity of the majority of paternally inherited alleles is absent or strongly reduced during the first divisions of the zygote. We expect that there is gene-to-gene and embryo-to-embryo variation (Vielle-Calzada et al. 2001), but a strong non-equivalence in allelic expression levels has been found for the vast majority of loci that were studied. For instance, additional supporting evidence for the non-equivalence of maternal and paternal genomes during early seed development has been generated by reporter gene analyses (e.g., Luo et al. 2000; Springer et al. 2000; Baroux et al. 2001; Sørensen et al. 2001; Golden et al. 2002) and more recently in maize by microarray analyses (Grimanelli et al. 2005). This characteristic of sexual seed development may allow the adaptive ability of apomictic species to reproduce without paternal contribution. The wide-spread absence of paternal activity during early seed development may not be a characteristic of all species, and the extent of this phenomenon may vary, as significant numbers of both paternally silent and bi-allelically active loci have been reported in maize (Grimanelli et al. 2005; Meyer, Scholten 2007). Thus, the successful introduction of apomixis into sexual species may also depend on certain aspects of sexual reproduction, which could be species-specific.

In addition to the *Arabidopsis msi1* mutant, which produces a few non-viable parthenogenetic embryos, there are several mutants that produce a higher number of viable haploid offspring. Among these, the maize mutant *indeterminate gametophyte 1* (*ig1*) leads to a four- to five-fold increase in maternal haploid production (Kermicle 1969). *Ig1* was recently shown to encode a LOB-domain protein (Evans 2007) that belongs to a family of plant-specific transcription factors (Husbands et al. 2007). *Ig1* controls free nuclear cell divisions in the female gametophyte (Lin 1978, 1981) and produces a wide variety of phenotypes, including additional egg and synergid cells, as well as extra nuclei in the central cell. How this gametophytic phenotype is related to the production of maternal haploids and the high frequency of paternal haploids in *ig1* mutants, up to 5% as compared to 0.001% in the wild type (Kermicle 1969), is not well understood. Extremely high frequencies of maternal haploid production have also been reported in barley plants carrying the *haploid inducer* (*hap*) mutation. Plants homozygous for *hap* produce up to 30% parthenogenetic offspring (Hagberg and Hagberg 1980; Asker et al. 1983), but neither the cellular events underlying this phenomenon nor the molecular nature of the *hap* mutation are known. While the central cell is fertilized normally, the sperm is prevented from fusing with the egg cell through an unknown mechanism (Mogensen 1988).

There are several other mechanisms that lead to the production of haploid offspring, but they rely on more complex genetic interactions. Inter-specific crosses can lead to the production of haploid offspring, e.g. in the crosses *Solanum tuberosum* × *Solanum phyreja* (Peloquin and Hougas 1959) or *Triticum aestivum* × *Pennisetum glaucum* (Zenkteler and Nitzsche 1984). Although this method is widely used for double-haploid production in plant breeding, it is of lesser interest with respect to this review, because it does not depend on autonomous egg activation. In fact, double fertilization still occurs in these crosses, but the paternal chromosome set is eliminated later on during embryogenesis (Gernand et al. 2005). Induced parthenogenesis, however, can also occur in intra-specific crosses. Certain genotypes of maize can induce a high frequency of maternal haploids if used as pollen donors. For instance, maize Stock 6 has an induction rate of 2.3% (Coe 1959; Sarkar and Coe 1966) and derivatives of this stock can induce haploids at frequencies of over 8% (Röber et al. 2005). Genetic studies have identified two major Quantitative Trait Loci in Stock 6 that are involved in haploid induction (Deimling et al. 1997), but the exact cellular events involved in this interesting phenomenon remain unclear.

By far the most efficient system for maternal haploid production is the "Salmon'' system of wheat with an occurrence of up to 90% parthenogenesis from the reduced egg cell (reviewed in Matzk 1996; Tsunewaki and Mukai 1990). The genetic control of this system is complex. It involves the translocation of the long arm of wheat chromosome 1B with the short arm of rye chromomsome 1R. This translocation provides a specific combination of nuclear factors characterized by the absence of the *Restorer of fertility* (*Rfv1*) locus and the *Suppressor of parthenogenesis* (*Spg*) locus from wheat and the presence of the *Parthenogenesis* (*Ptg*) locus from rye (see Fig. 2 for details). However, this complex genotype will result in parthenogenesis only if combined with the cytoplasm of *Aegilops caudata* or *Aegilops kotschyi*, demonstrating the importance of nuclear-cytoplasmic interactions. Importantly, cultivated Salmon-wheat egg cells can develop autonomously into parthenogenetic embryos (Kumlehn et al. 2001), illustrating that no external signals are required and that all factors required for egg activation are present in the quiescent egg cell. While the exact cellular events that lead to egg activation and embryogenesis in Salmon wheat are not known, the system offers a unique opportunity to analyze the first steps of parthenogenesis at the molecular level (Balzer et al. 1996; Matzk et al. 1995, 1997, 2007).

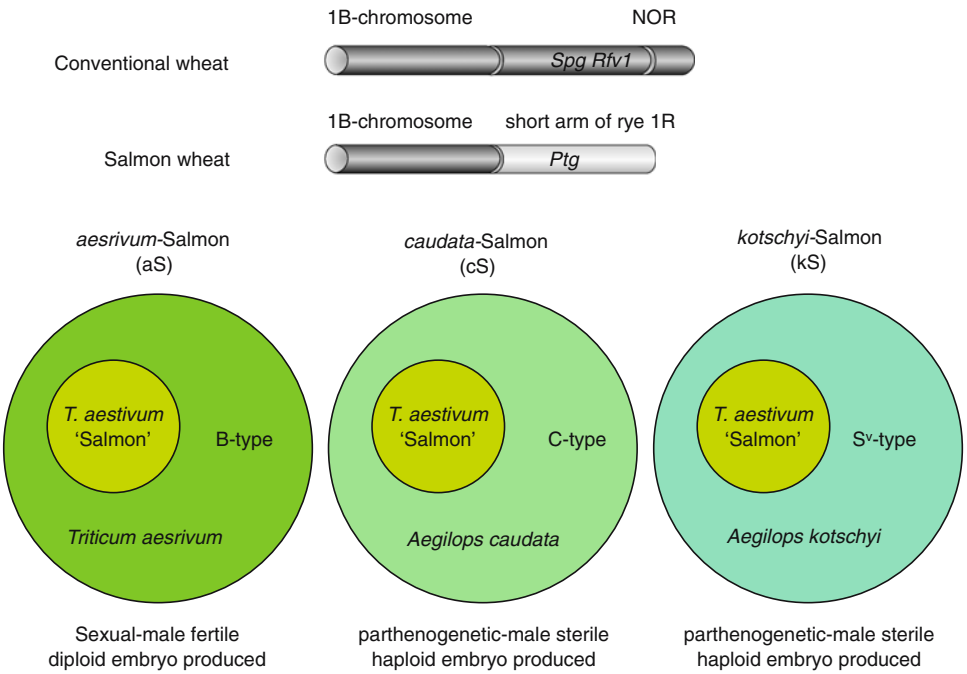

Fig. 2 Parthenogenetic "Salmon'' system wheat. "Salmon'' wheat contains a translocation that replaces the short arm of chromosome 1B with the short arm of rye chromosome 1R. This translocation removes the *Rfv1* locus and the Nucleolar Organizer Region (NOR) situated on this arm. The *Rfv1* locus is capable of restoring fertility to various *Aegilops* cytoplasm-types, including the S^v-type. This translocation also replaces a *suppressor of parthenogenesis* gene (*Spg*) with the *parthenogenesis* gene (*Ptg*). "Salmon'' wheat lines that contain a combination of the long arm of wheat chromosome 1B and the short arm of rye chromosome 1R together with a parthenogenesis inducing cytoplasm of *Aegilops* exhibit autonomous embryo development. Seed development depends, however, on the fertilization of the polar nuclei (i.e. pseudogamy). NOR (Nucleolar organizing region), *Spg* (*suppression of parthenogenesis*), *Ptg* (*parthenogenesis*), *Rfv1* (*fertility restoring gene*), C-type and S^v-type cytoplasm (parthenogenesis inducing), B-type cytoplasm (non-parthenogenesis inducing)

Conclusions

Experimental approaches to dissect sexual reproduction in plants, as described in this review, have revealed some of the controlling elements that prevent precocious seed development in the absence of fertilization. While these approaches have provided much insight into our understanding of sexual reproduction, we are far from being able to utilize this knowledge to develop apomixis as a technology capable of harnessing the benefits of fixing complex heterozygous genotypes, including those of F1 hybrids of crop plants. Although the early steps toward this goal are underway, many new technologies must be developed and exploited if this major challenge in plant biotechnology is to be achieved. An integrative approach that makes use of both apomictic and sexual model plant systems will permit new insights into the precise molecular technology required to engineer synthetic apomictic crops. The application of new technologies, such as laser-assisted microdissection (LAM) (Day et al. 2005; Casson et al. 2005) or novel gain-of-function screening approaches (Brand et al. 2006) may play a major role in these efforts. Although traditional loss-of-function mutations have, so far, played a major role in the molecular dissection of sexual reproduction, future breakthroughs are likely to emerge from novel cell-type specific activation tagging approaches that permit the initiation of unreduced embryo sac development, the spontaneous activation of an egg cell, or that allow autonomous endosperm development.

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